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Lipo-oligosaccharides of *Campylobacter jejuni* serotype O:10. Structures of core oligosaccharide regions from a bacterial isolate from a patient with the Miller–Fisher syndrome and from the serotype reference strain

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Abstract

Lipo-oligosaccharide (**LOSa**) was obtained by phenol–water extraction of bacterial cells of an isolate PG 836, identified as *Campylobacter jejuni* serotype O:10, from a patient who subsequently developed the Miller–Fisher syndrome (MFS). The product was separated into a water-insoluble gel of low M_r and a water-soluble component of high M_r. The structure of the core oligosaccharide region in **LOSa** is reported herein for comparison with **LOSb** from the *C. jejuni* O:10 reference strain, and is based on investigations carried out on: (1) *O*-deacylated **LOSa**; (2) the core oligosaccharide (**OS 1a**) liberated on acetic acid hydrolysis of the ketosidic linkages to lipid A, with accompanying loss of *N*-acetylneuraminic acid residues; (3) the product of the removal of phosphate residues from **OS 1a** to give **OS 2a**; and (4) the Smith degradation of **OS 2a** to yield a mixture of **Os 3a** and **OS 4a**. The results revealed that the core oligosaccharide region in **LOSa** from the MFS bacterial isolate had chains (**1a**), of which some were terminated by an *N*-acetylneuraminobiose [Neu5Ac(α2–8)Neu5Ac] unit in a GD_3 [Neu5Ac–Neu5Ac–Gal] epitope, and the inner regions of which were different from those of other *C. jejuni* serotypes. Similar experiments on **LOSb** from bacterial cells of the *C. jejuni* O:10 reference strain showed that the core oligosaccharide unit

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[**1a**, R = P (phosphoric monoester)] of **LOSa** from the MFS isolate was more uniformly complete than that of the O:10 reference strain [**1b**, R = AEP (2-aminoethylphosphate)] differing in the nature of the phosphate substituent at the inner heptose residue. The close structural relationship of **LOSa** from the MFS associated bacterium to **LOSb** from the O:10 reference strain runs parallel to that of the previously studied Guillain–Barré syndrome (GBS) associated bacterium typed as *C. jejuni* O:19 in comparison with the lipo-oligosaccharide from the reference strain. Preliminary studies on the high M_r components showed that those from the O:10 strains were indistinguishable from each other, but were structurally unrelated to those from the GBS associated *C. jejuni* serotype O:19 isolates and the O:19 reference strain [G.O. Aspinall, A.G. McDonald, and H. Pang, *Biochemistry*, 33 (1994) 250–255].

Keywords: *Campylobacter jejuni* serotype O:10; Lipo-oligosaccharide; GD_3 epitope; *N*-acetylneuraminobiose units; Guillain–Barré syndrome; Miller–Fisher syndrome

1. Introduction

The Miller–Fisher syndrome (MFS) is an infrequent variation of the more common neuropathy known as the Guillain–Barré syndrome (GBS). The onset of GBS is often preceded by intestinal infections with *Campylobacter jejuni*. Kuroki et al. [1] reported that 10 of their 14 cases of GBS were preceded by diarrhoea due to *C. jejuni* of serotype O:19. In previous investigations, lipopolysaccharides of low M_r, now designated as lipooligosaccharides (LOSs) from two GBS-related isolates, OH 4382 and OH 4384 typed as *C. jejuni* O:19, were compared with that from the O:19 reference strain [2–4]. The high M_r components were indistinguishable, with polysaccharide chains consisting of disaccharide repeating units of an amidated hyaluronic acid. The core oligosaccharide (OS) regions in the O:19 reference strain consisted of molecules with terminal units that mimic human gangliosides G_{D1a} and G_{M1}. The core OS regions of the OH4382 and OH4384 LOSs differed in structure, but both contained a terminal trisaccharide epitope, Neu5Ac(α2–8)Neu5Ac(α2-3)Galβ, in mimicry of the human ganglioside GD_3. When a bacterial isolate was obtained from a patient with MFS at the Rhode Island Hospital, Providence, RI, USA, the opportunity was taken to carry an examination of **LOSa** isolated from this initially untyped *C. jejuni* strain PG 836 (Fig. 1). Subsequent serotyping and immunoblotting evidence [5] revealed that the isolate was not that of serotype O:19, but belonged to a different serotype, O:10, whose lipopolysaccharides had not been previously studied. The examination of **LOSb** from the O:10 reference strain, which was initiated after that of the PG 836 strain was nearly complete, is reported in this paper in sufficient detail to establish any differences in structure, but without the unnecessary repetition of identical results. An account of the isolation of **LOSa** from the MFS associated strain has been published with some preliminary results of its structural examination [6]. A *C. jejuni* isolate associated with the same neuropathy, typed as O:2, has been reported [7] but, to our knowledge, no detailed chemical studies have been described.

2. Experimental

Extraction of LOSs from cells of C.jejuni *strain PG 836 and the O:10 reference strain, and preparation of derived oligosaccharides* **OS 1a** and **OS 1b**.—Details of the isolation, etc. of *C. jejuni* strain PG 836 have been published [6] and bacterial cells of the O:10 reference strain were similarly obtained. For both strains, phenol–water extractions furnished lipo-oligosaccharide (LOS) as a water-insoluble gel which was deposited on ultracentrifugation (100,000 × *g* for 4 h) and polysaccharide of high M_r which was obtained from the supernatant solution. A portion of the insoluble gel was treated with anhydrous hydrazine at 40 °C for 1 h to yield *O*-deacylated LOS. The remainder of the gel-like pellet was hydrolysed in aqueous 1% acetic acid for 1 h at 100 °C. Insoluble lipid **A** was removed by centrifugation. Water-soluble material was fractionated by gel permeation chromatography on Bio-Gel P-2 to give **OS 1**.

Analytical procedures.—GLC was carried out on a Hewlett–Packard model 5890A chromatograph. Separations were carried out using capillary columns with the following programs: DB-23 (30 m × 0.25 mm) (A) isothermally at 220 °C, (B) isothermally at

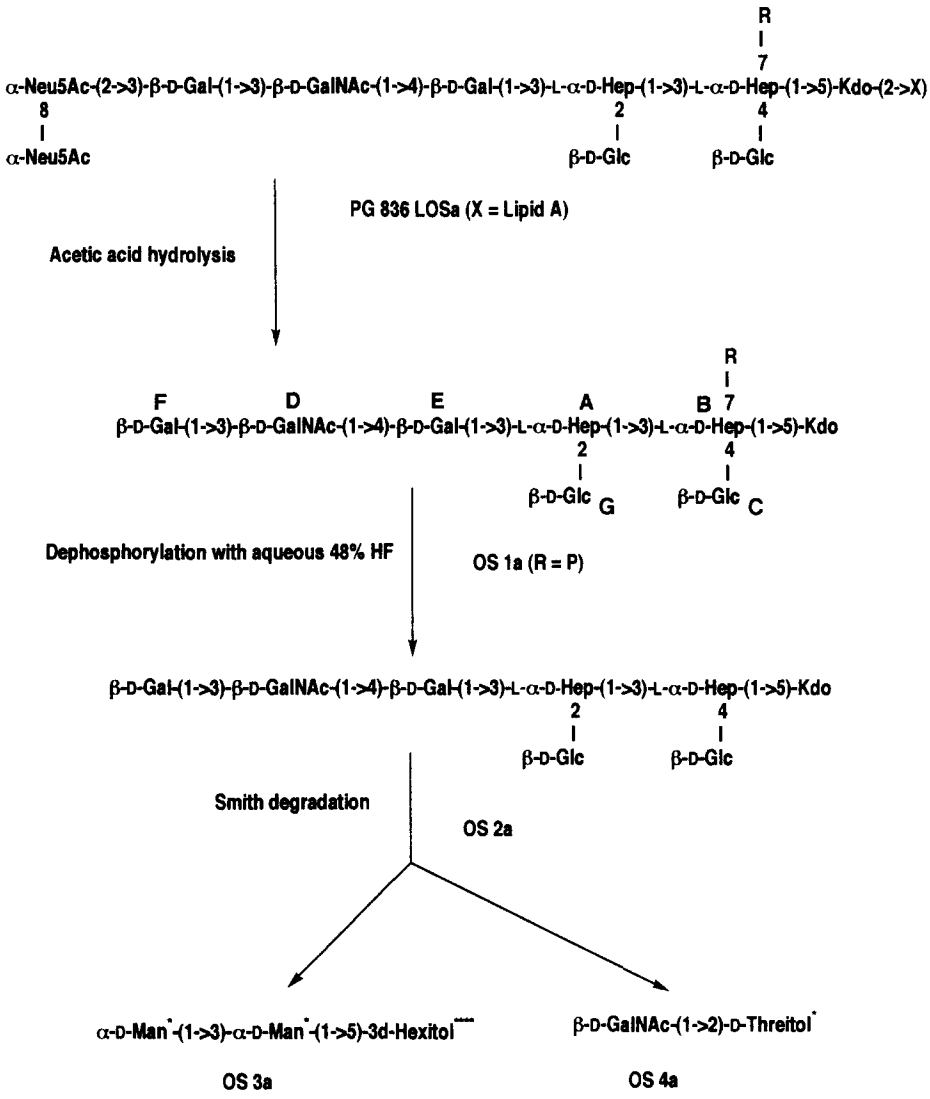


Fig. 1. Genesis of OS fractions from the *C. jejuni* PG 836 **LOSa**. 3d-Hexitol = 3-deoxyhexitol. Asterisks show incorportion of the ^{2}H isotope.

190 °C, (C) at 190 °C (10 min), 200 °C to 250 °C at 2°/min; DB-17 (15 m × 0.25 mm) (D) isothermally at 190 °C; DB-5 (15 m × 0.25 mm) (E) from 180 °C to 230 °C at 3°/min. EI GLC–MS was performed using a KRATOS Analytical Profile instrument with electron impact ionisation at 70 eV.

FAB mass spectra were acquired as described in previous papers [3,8]. Interpretations of positive ion mass spectra of permethylated derivatives were as described by Dell et al. [9] and used in earlier publications [3,8]. Spectra of methylated derivatives from OS with reducing Kdo termini showed that the major component of the molecular ion cluster corresponded to that calculated for $[M + H]^+$ or other adduct ions. Related ions at $([M + H]^+-46)$ and $([M + H]^+-116)$ were sometimes present and their co-occurrence aided in the identification of molecular ions.

Other analytical procedures.—Glycose analyses as alditol acetate derivatives were performed by GLC using program A. Methylations of oligo- and polysaccharides (0.3–0.5 mg) were performed in dimethyl sulfoxide by the method of Ciucanu and Kerek [10] as described previously [3,8]. Enantiomeric configurations were established by conversion into 2-(*R*)- and 2-(*S*)-butyl glycosides [11] and GLC analysis of acetylated derivatives using program D. The identities of Kdo and Neu5Ac derivatives in core **OS**, *O*-deacylated **LOS**, and their methylated derivatives

were established by methanolysis (0.75 M CF_3CO_2H at 100 °C for 6 h), followed by acetylation and GLC–MS analysis using program E.

Miscellaneous degradations.—*O*-Dephosphorylations were carried out by treatment with 48% aqueous hydrogen fluoride at 4 °C for 48 h. Excess of hydrogen fluoride was removed in a vacuum desiccator over KOH, and the products were fractionated by GPC on Bio-Gel P-4. For the Smith degradation, core oligosaccharides (~4 mg) were prereduced with $NaBD_4$ (3 mg) in water (4 mL) at room temperature for 12 h. The oligosaccharide alditols were successively oxidized with $NaIO_4$, as described by Pritchard et al. [12], desalted by GPC on Bio-Gel P-2, reduced with $NaBD_4$, decationized and concentrated with methanol, hydrolyzed with 1 M CF_3CO_2H at room temperature for 3 h, and aldonolactones (from modified Kdo residues) were reduced by treatment again with $NaBD_4$ [3], and isolated by GPC on Bio-Gel P-2.

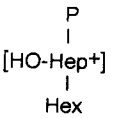
^{1}H and ^{31}P NMR spectroscopy.—One-dimensional (1D) and two-dimensional (2D) experiments were performed on a Bruker ARX 400 spectrometer in the Fourier transform mode. Oligosaccharide and glycan samples were exchanged several times with deuterium oxide (99.8%). Chemical shifts were measured relative to internal acetone, δ 2.225 for ^{1}H NMR. Chemical shifts for ^{31}P NMR were measured with reference to external orthophosphoric acid (δ 0.0).

2D Experiments were performed with the following parameters: COSY, COSYRCT, and TOCSY [512 × 1024 data matrix, zero-filled to 1024 data points in t_1, spectral width of 2008 Hz, 64 or 72 scans per t_1 value, 1.0 or 1.5 s for the recycle delay, mixing time of 80 ms for TOCSY, unshifted sine-bell filtering in t_1 and t_2 for COSY and COSYRCT, but shifted sine-squared filtering in t_1 and t_2 for TOCSY as for all phase-sensitive experiments]; for NOESY [256 × 2048 data matrix, zero-filled to 1024 data points in t_1, 128 scans per t_1 value, recycle delay of 2.0 s]; for ^{1}H–^{1}H ROESY [1024 × 1024 data matrix, mixing time of 250 ms, phase sensitive using TPPI]. 1D TOCSY experiments were performed using the same parameters as for 2D TOCSY [13].

3. Results and discussion

LOSa from the PG 836 strain was isolated as a gel-like pellet by ultracentrifugation of the aqueous layer from the phenol–water extraction. Fig. 1 summarizes the structures developed from experiments performed on **LOSa** after *O*-deacylation, and on the following core OS derivatives: **OS 1a** from treatment of **LOSa** to cleave the linkage from Kdo to lipid **A** with accompanying hydrolysis of Neu5Ac linkages; **OS 2a** from **OS 1a** on *O*-dephosphorylation; and **OS 3a** and **OS 4a** formed as a mixture of components from **OS 2a** on subjection to the Smith degradation with introduction of isotopic [^{2}H]-labelling in the reduction steps [3]. Compositional analysis of core oligosaccharides **OS 1a** and **OS 2a** after successive removals of neuraminic acid units and phosphate showed the presence of Gal, Glc, GalNAc, and LD-Hep residues in the approximate proportions of 2:2:1:2. All sugars had the D-enantiomeric configuration as shown by the formation of acetylated chiral 2-butyl glycosides [11]. Qualitative evidence for the presence of Neu5Ac and Kdo residues was obtained through formation of methyl ester methyl glycosides. The presence of a Kdo terminus was inferred from fast atom bombardment–mass spectrometric (FABMS) data in studies on permethylated oligosaccharides **OS 1a**, **OS 2a**, and **OS 3a**, and similarly, linkage analysis and the associated FABMS showed terminal Neu5Ac residues. A single phosphoric ester substituent was detected by ^{31}P NMR spectroscopy.

Composition, linkage, and sequence determination of oligosaccharides **OS 1a** *and* **OS 2a**, *and for the complete core oligosaccharide region in the LOS.*—The FAB mass spectrum of permethylated **OS 1a** (Fig. 2) gave pseudomolecular ions $[M + Na]^+$ at *m/z* 1996, $[(M + Na)-46]^+$ at *m/z* 1950, and $[(M + Na)-116]^+$ at *m/z* 1880, corresponding to a composition of Hex_4 Hep_2 HexNAc Kdo and a phosphoric monoester substituent. Glycosyloxonium fragment ions at *m/z* 464 [Hex–HexNAc] and 1120 [Hex–HexNAc–Hex–(Hex)Hep] (Fig. 3), taken together with methylation linkage analysis data (Table 1), were consistent with the outer portion of the proposed structure for **OS 1a**. A further ion at *m/z* 1720 was consistent with that for a pseudomolecular ion $[M + Na]^+$ for a methyl glycoside of a Kdo-deficient degradation product with the inner Hep residue bearing Hex and phosphate substituents. The presence of this inner phosphorylated Hep residue was also indicated by the detection, albeit in weak abundance, of a double cleavage ion in the FABMS for **OS 1a** at *m/z* 547. Evidence for attachment of



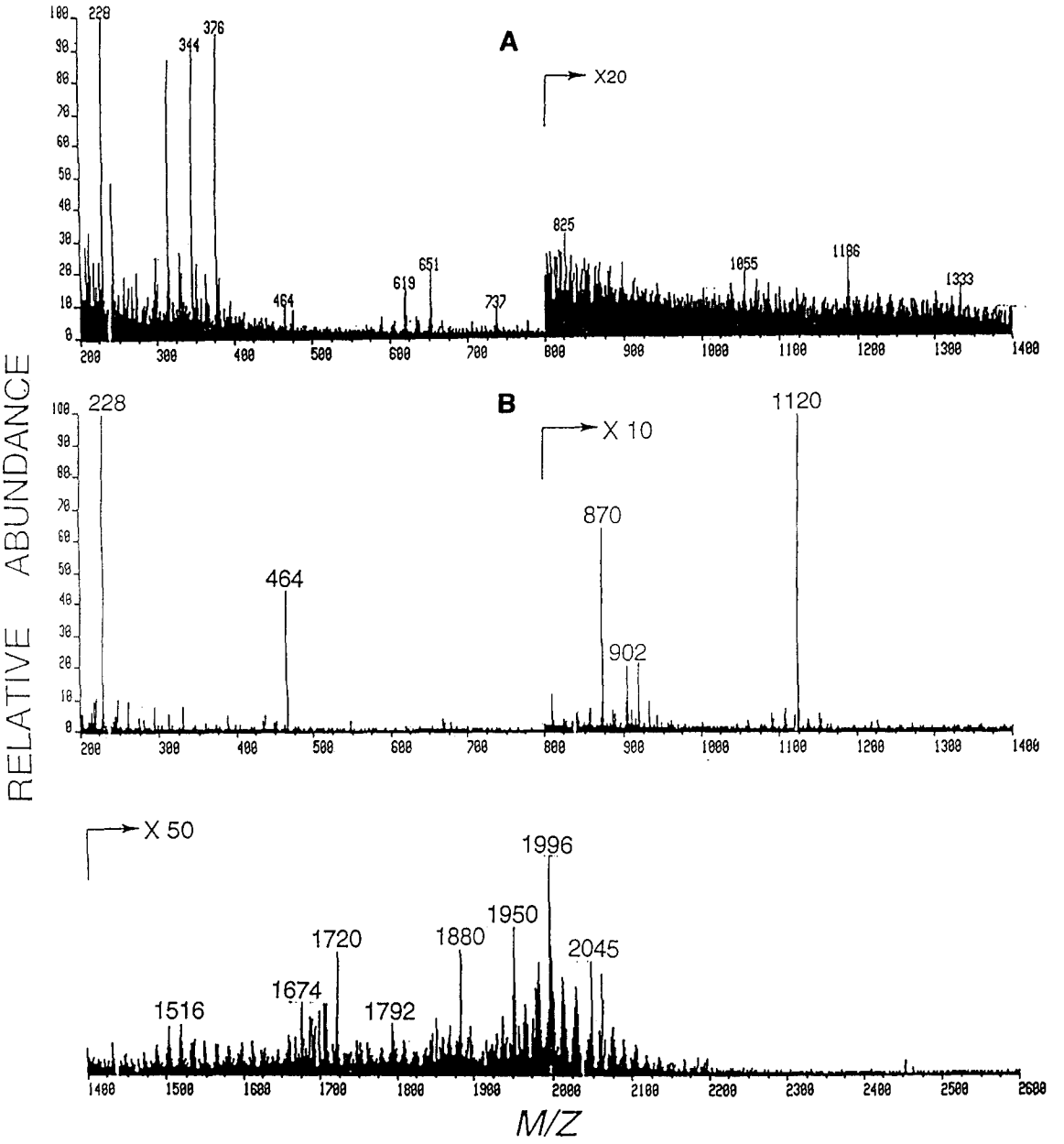


Fig. 2. Positive ion FABMS spectra of (A) permethylated **LOSa** and (B) **OS 1a** from the *C. jejuni* PG 836 strain.

the phosphoric ester at O-6 was obtained when *O*-dephosphorylation of permethylated **OS 1a** was followed by linkage analysis to give 2,7-Me_2 Hep in addition to those liberated on direct hydrolysis of methylated **OS 1a**. The corresponding residue on hydrolysis of permethylated **OS 2a** (Table 1) furnished 2,6,7-Me_3 Hep. FABMS of permethylated **OS 2a** showed pseudomolecular ions at $[M + Na]^+$ at *m/z* 1902, $[(M + Na)-46]^+$ at *m/z* 1856, and $[(M + Na)-116]^+$ at *m/z* 1786, and the same two fragment ions at *m/z* 464 and 1120 as from methylated **OS 1a**, in confirmation of the attachment of phosphate on the inner Hep residue.

When the PG 836 **LOSa** preparation was heated with 1% acetic acid to liberate the core OS region, complete loss of Neu5Ac residues occurred. This was in contrast to the behaviour of the GBS-related strains and the serostrain from *C. jejuni* O:19 [3], as also of the LOS from the *C. jejuni* O:4 reference strain [8]. In each of these instances the mild acid treatment had resulted in loss of the terminally attached Neu5Ac residues, but much of the Neu5Ac linked laterally to an internal Gal residue remained attached. To obtain information on the nature and location of these acid-sensitive residues, the *O*-deacylated PG 836 LOS, from treatment with hydrazine, was permethylated.

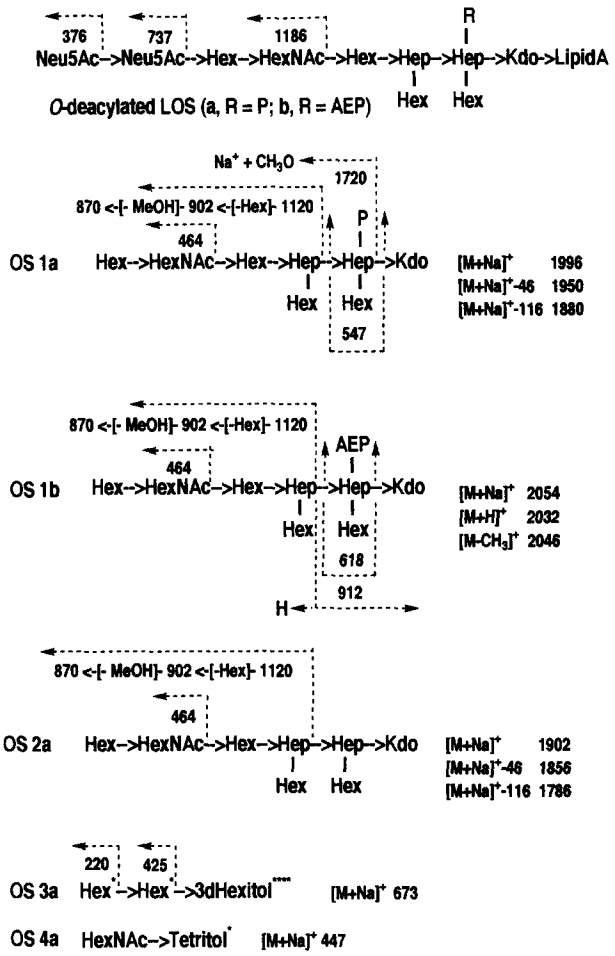


Fig. 3. Analysis of positive ion FABMS data for permethylated **LOSa**, **OS 1a**, **OS 2a**, **OS 3a** and **OS 4a** from the *C. jejuni* PG 836 strain, and **LOSb** and **OS 1b** from the *C. jejuni* O:10 reference strain.

FABMS data (Fig. 2, panel A, and Fig. 3) showed fragment ions at *m/z* 376 (Neu5Ac) and 737 ($Neu5Ac_2$) for monomeric and dimeric units, and the corresponding units in attachment to Hex–HexNAc units at *m/z* 825 and 1186. Linkage analysis (Table 1) showed that *O*-3 of the Gal end group in **OS 1a** and **OS 2a** was the only site of attachment of both mono- and disialyl units. Linkage analysis from methanolysis of the permethylated LPS giving 4,7,8,9-Me_4 and 4,7,9-Me_3 derivatives provided evidence for a (2–8) linkage in the Neu5Ac biose units [14], and hence for the presence of the terminal trisaccharide epitope, Neu5Ac(α2–8)Neu5Ac(α2–3)Galβ, in mimicry of the human ganglioside GD_3. The foregoing experiments showed that of the three undifferentiated Hex end groups in **OS 1a**, that of Gal from removal of Neu5Ac residues was at the chain terminus, and hence that the two Glc end groups were linked, one to each, to the two inner Hep residues. This Gal end group represented the sole point of attachment of Neu5Ac residues, with none linked laterally to the inner Gal residue. In a search for further linkage information in the inner core region, a Smith degradation was carried out on **OS 2a** which had been reduced with $NaBD_4$, and the sequence of reactions of oxidation, reduction ($NaBD_4$), and hydrolysis of acyclic acetals accompanied by lactonization, was followed by a further reduction ($NaBD_4$) leading to a mixture of products, **OS 3a** and **OS 4a**. Despite incomplete separation of **OS 3a** and **OS 4a**, linkage analysis and FABMS of the methylated derivatives were adequate to define linkage sequences. Thus the detection of fragment ions at *m/z* 220 and 425 and a pseudomolecular ion $[M + Na]^+$ at *m/z* 673 for **OS 3a** was consistent with the presence of two [$^2H_1$] Man residues resulting from attenuation of the LDHep residues and a 3-deoxy-[$^2H_4$]-hexitol unit originating from the degradation of a 5-linked Kdo moiety [3,7]. Linkage analysis identified substitution sites and the positions of isotopic incorporation. The presence of **OS 4a** in the mixture of products from the Smith degradation was indicated by the detection by FABMS of a pseudomolecular ion $[M + Na]^+$ at *m/z* 447 and the formation of *N*,3,4,6-Me_4 GalNAc on hydrolysis, despite failure to detect the easily lost volatile Me_3 [$^2H_1$]-tetritol. The characterization of OS **3a** provided evidence for a Hep-(1–>3)–Hep-(1–>5)-Kdo in the inner core region. With the previous location of phosphate at O-7 of the inner Hep residue the presence of a Glc residue at O-4 could be inferred, but differentiation of sites of chain extension and attachment of the second Glc residue at O-3 and O-2 of the outer Hep residue remained to be achieved. An attempt to answer this question by digestion of **OS 2a** with β-D-glucosidase was unsuccessful. The oligosaccharide was recovered unchanged except for loss of the terminal Gal residue, presumably due to the action of β-D-galactosidase as a minor impurity in the enzyme preparation.

Configurational assignments in **OS 1a** *and* **OS 2a** *by* ^{1}H *NMR spectroscopy and differentiation of linkage sites at branch points.*—^{1}H NMR data showed the presence in both **OS 1a** and **OS 2a** of seven anomeric protons, of which two at δ 5.25 and 5.05 as unresolved doublets characteristic of the α-D-*manno* configurations of LD-Hep residues, and the others with coupling constants $J_{1,2} \sim 8$ Hz were of β-D-hexopyranosides with the *gluco* or *galacto* configurations of Glc, Gal or GalNAc residues and, with one exception in **OS 1a**, were in the δ 4.4–4.7 range. The ring configurations for individual residues were

Table 1
Linkage analysis of methylated derivatives from LOSs of *C. jejuni* strain PG 836 and serotype O:10

Structural units from methylation analysis	Approximate relative proportions in oligosaccharides[a]							
	PG 836	**LOSa**	**OS 1a**	**OS 2a**	**OS 3a**	**Serotype O:10**	**LOSb**	**OS 1b**
Glc		2	2	2			2	2
Gal		tr	1	1			tr	1
→ 3-Gal		1					1	
→ 4-Gal		1	1	1			1	1
→ 3-GalNAc		1	1	1			1	1
Man*					1			
→ 3-Man*					1		1	
→ 2,3-Hep		1	1	1			1	1
→ 3,4-Hep				1				
→ 3,4,6-Hep				1[b]			tr	
→ 3-d Hexitol*[c] [from → 5 Kdo]				tr				
→ 5-d Octitol[d]								1
Neu5Ac[e]		1					1	
→ 8-Neu5Ac[e]		1					1	

[a]Molar ratios are based on relative peak areas in GC/MS analyses of partially methylated alditol acetates and normalized with respect to Gal or Man derivatives.
[b]Detected after dephosphorylation of methylated **OS 1a**.
[c] → 3-d Hexitol, 3-deoxyhexitol.
[d] → 5-d Octitol, 3-deoxyoctitol.
[e]Neu5Ac derivatives were identified by GC/MS of methyl ester methyl glycosides.
* Denotes ^{2}H incorporation from reduction with $NaB[^{2}H_4]$.

determined through 2D COSY and TOCSY experiments to a sufficient extent to distinguish between *galacto* of Gal or GalNAc, *gluco* of Glc, and *manno* of LD-Hep configurations as a basis for assigning ring protons of residues at branch points (Table 2). Residue **C** with an anomeric proton at δ 4.82 ($J_{1,2}$ = 7.9 Hz) had the β-D-*gluco* configuration in **OS 1a** and was attached to O-4 of the inner Hep residue. When **OS 1a** was *O*-dephosphorylated, the anomeric proton of the corresponding residue in **OS 2a** had δ 4.49 ($J_{1,2}$ = 8.0 Hz) and no other changes in the ^{1}H NMR spectra were observed. The assigned resonances of the ring protons of residues in **OS 1a** and **OS 2a** provided the basis for the interpretation of inter-residue NOE interactions in a ROESY experiment. The previously unresolved linkages at the outer L-α-D-Hep residue **A** were differentiated by the detection of connectivities from H-1 of β-D-Gal residue **E** at δ 4.56 to H-3 of residue **A** at δ 4.15, and from H-1 of β-D-Glc residue **G** at δ 4.33 to H-2 of residue **A** at δ 4.30. The detection of an NOE interaction from H-1 of residue **D** at δ 4.65 to H-4 of residue **E** at δ 4.05 confirmed the identity of residue **D** as GalNAc. With incomplete assignments of ^{1}H resonances in the Kdo

Table 2
Selected ^{1}H NMR chemical shift (ppm) and coupling constant data (in parenthesis $J_{n,n+1}$ in Hz) from 2D COSY and 1D TOCSY for **OS 1a** from strain PG 836 and **OS 1b** from LOS of the O:10 reference strain

Residue	Assigned ring configuration	H-1	H-2	H-3	H-4
A	α-Man [of LDHep]	5.25 (bs)	4.30 (3.1)	4.15 (~7)	3.93 (~9)
B	α-Man [of LDHep]	5.05 (bs)	4.10 (s)	3.95 (~7)	3.53 (~9)
C	β-Glc	4.82 (8.2)	3.24 (8.8)	3.49 (8.8)	3.14 (9.8)
		4.49[a] (8.0)			
D	β-GalNAc	4.65 (4.0)	3.95 (7.5)	3.85 (2.5)	4.10 (ud)
E	β-Gal	4.56 (7.8)	3.45 (9.3)	3.73 (1.9)	4.05 (5.0)
F	β-Gal	4.40 (7.9)	3.49 (9.9)	3.59 (2.8)	3.87 (4.5)
G	β-Glc	4.33 (7.7)	3.27 (8.2)	3.44 (8.7)	3.69 (10.8)

bs = Broad signal (unresolved doublet).
The data from the two strains differed by < 0.02 ppm and are not duplicated.
[a]Only the H-1 resonance of residue **C** was altered on *O*-dephosphorylation of **OS 1**.

residue in **OS 1a** and **OS 2a**, it was not possible to obtain NOE data for inter-residue connectivity of H-1 of residue **B** to the Kdo residue in support of the chemical evidence from the Smith degradation of **OS 2a** (Fig. 1) for a 5-linked terminus.

O-*Deacylated* **LOSb** *from the* C. jejuni *O:10 reference strain, and core oligosaccharides* **OS 1b** *and* **OS 2b**.—A preliminary examination of the initially isolated water-insoluble gel from the reference strain had indicated some difference from the PG 836 strain and a degree of heterogeneity as revealed in the banding patterns shown on SDS–PAGE with detection by silver staining or by immunoblotting with the O:10 serotyping antiserum [6]. Heating with 1% acetic acid to liberate the core OS region resulted in complete loss of Neu5Ac residues. It had been anticipated that the differences in banding patterns between the *C. jejuni* O:10 reference strain **LOSb** and that of **LOSa** from the PG 836 strain might reflect less complete sialylation in the former than in that from the neuropathy strain, in similar fashion to the *C. jejuni* O:19 reference strain in relation to the O:19-typed isolates from Guillain–Barré syndrome patients [3]. However, the results showed that this was not the case. Linkage analysis indicated the formation of terminal and 8-linked Neu5Ac derivatives in close to equimolar proportions, and FABMS data showed the same fragment ions as in the PG 836 strain (Figs. 2 and 3) pointing to termination of the majority of chains by a neuraminobiose unit. Compositional analysis of **OS 1b** liberated from the gel on mild acetic acid treatment with a shortfall in the relative proportions of Gal and GalNAc suggested the presence of incomplete chains in the original **LOSb** preparation. Linkage analysis (Table 1) in conjunction with the FABMS of permethylated **OS 1b** (Fig. 3) supported this conclusion. The major ion clusters showed pseudomolecular ions for $[M + Na]^+$, $[M + H]^+$, and $[M - CH_3]^+$ at *m/z* 2054, 2032 and 2046 corresponding to a composition of Hex_4 Hep_2 HexNAc AEP Kdo, and the less abundant cluster showed pseudomolecular ions at *m/z* 1605, 1583, and 1597 corresponding to an [M-(Hex + HexNAc)] species. The fragment ions and the linkage analysis (Table 1) showed that the outer chains inclusive of Hep residue **A** and attached Glc residue **G** in the asialo **OS 1b** from the O:10 reference strain contained identically linked sugar residues to those in the corresponding PG 836 asialo **OS 1a** (Fig. 2, panel B and Fig. 3). Fig. 3 shows the proposed structure for the complete **OS 1b**. Characteristically no glycosyloxonium ion was detected from cleavage at the phosphate-bearing Hep residue. However, both a double cleavage ion at *m/z* 618 and a 'reducing end' ion at *m/z* 912 from B pathway cleavage shown in Fig. 3 furnished evidence for the location of the 2-aminoethylphosphate (AEP) substituent on the inner Hep residue bearing an attached Hex (Glc) residue as a structural difference between the O:10 reference strain and the PG 836 strain. The site of linkage of the inner Hep residue to Kdo was established using the procedure of Tacken et al. [15] in which the terminal residue in methylated **OS 1b** was shown to be 5-substituted (Table 1). During this analysis the permethylated derivative was *O*-dephosphorylated, and hydrolysis followed by derivatization showed the inner phosphorylated heptose residue to have been 3,4,7-tri-*O*-substituted. With confirmation from NMR data (below) of the glycosylation sites at O-3 and O-4, this evidence pointed to phosphorylation at O-6, as in the corresponding methylated **OS 1a** derivative from the PG 836 strain. In the absence of ^1H assignments at H-6 and H-7 of Hep residues as the basis for the detection of ^1H–^{31}P connectivities, no independent evidence for the location of the different phosphate substituents in the **OS 1a** and **OS 1b** samples could be obtained. There was however, no indication of possible phosphate migration from O-6 to O-7 during the methylation of phosphoric diesters, as reported by Holst and collaborators [16].

^1H NMR data for the ring proton resonances in **OS 1b** and **OS 2b** from the reference strain were superimposable on those of the corresponding compounds from the PG 836 strain. The same downfield shift was shown by H-1 of the β-D-Glc residue **C** at δ 4.82 for **OS 1b** as in the corresponding **OS 1a** from the PG 836 strain with a similar upfield shift of the H-1 resonance after *O*-dephosphorylation. Inter-residue connectivities in the NOESY spectrum confirmed the identity of sugar sequences to those in the PG 836 strain. Notable features in the spectrum of the **OS 1b** from the serostrain were the prominent resonances at δ 3.28 and 4.14 corresponding to the methylene groups of a 2-aminoethylphosphate unit between which a strong nOe was observed. These resonances disappeared on *O*-dephosphorylation of **OS 1b**.

Location of N-*acetylneuraminic acid residues in the lipo-oligosaccharide from the* C.jejuni *O:10 serostrain.*—Linkage analysis (Table 1) of *O*-deacylated **LOSb** and FABMS of the methylated derivative were performed as for the PG 836 strain. Terminal and 8-linked Neu5Ac residues liberated on methanolysis were identified, but in the absence of reference

samples quantitation could not be regarded as accurate. The absence of all but traces of the galactopyranose end groups of **OS 1b** and the appearance of 3-linked Gal residues pointed to the terminal Gal residues as the only site of sialylation and no evidence was obtained for laterally attached Neu5Ac residues at O-3 of the inner Gal residues. These results were consistent with the occurrence of the presence of chains with the neuraminobiose terminus as the dominant structure with only small proportions of chains carrying a single Neu5Ac residue.

The initially untyped *C. jejuni* PG 836 strain was obtained from a patient with MFS, a rather rare variant of GBS. The first structural study of the LOS component of this neuropathy-associated strain showed that, as for the two *C. jejuni* O:19-typed isolates from GBS patients, the oligosaccharide chain was terminated by the trisaccharide epitope, Neu5Acα2 → 8Neu5Acα2 → 3Galβ, in mimicry of the human ganglioside GD_3. Subsequently, the PG 836 strain was typed as *C. jejuni* O:10, a serotype of less common occurrence, whose LPSs had not been examined. The core oligosaccharide chains of the LOSs (LPSs of low M_r) from the PG 836 isolate and the O:10 serostrain proved to be identical in glycosyl composition and linkage type, and distinct from those of other known *C. jejuni* serotypes. In the O:10 and the two O:19-typed neuropathy related LOSs the *N*-acetylneuraminobiose unit is linked to the terminal β-D-Gal residue in the complete core OS chains for the PG 836 strain and the OH4384 isolate, and to a corresponding β-D-Gal residue in the incomplete chain in the OH4382 isolate. The essential requirements for the assembly of the GD_3 epitope, of a chain terminal β-D-Gal residue with O-3 able to accept the first Neu5Ac transfer, and the availability of a specific Neu5Ac2 → 8Neu5Ac transferase, would appear to be independent of the remaining core OS structure and of the O-serotype classification. With the discovery of the same GD_3 epitope in the O:10 serostrain, it would appear that this unit may be a necessary but not sufficient structural feature to distinguish neuropathogenic from non-neuropathogenic *C. jejuni* strains.

In comparison with other sialylated *C. jejuni* LPSs two further features may be noted. In the contrast to the O:19 strain [3] but as for the O:1 [8] and O:2 serotypes [17], the inner β-D-Gal residue in the core OS region of the O:10 LOS would appear unable to act as acceptor for a laterally attached Neu5Ac residue. The common feature in each of these instances is the presence of a β-D-Glc residue at O-2 of the distal LD-Hep residue. In common with the *C. jejuni* O:19 reference strain and the two O:19-typed isolates, LPS preparations from both O:10 strains contain a glycan of high M_r, for which there is no evidence of covalent connection as the O antigen component of an LPS. Preliminary studies of the glycan have shown it to contain two configurationally unknown 7-deoxyheptofuranose residues as single unit side-chain constituents of a glycosaminoglycan. The *C. jejuni* O:19 glycan, an unusual amidated derivative of hyaluronic acid [2,4], was present in each of the O:19-typed strains. With no differentiation between serostrain and GBS isolates, the possibility that this glycan might engage in a form of molecular mimicry of human hyaluronic acid and be a contributing factor in the development of a neuropathy was considered unlikely. The O:10 glycan is unrelated to other known glycans of high M_r and may be regarded as even less likely to be implicated in a mimicry role.

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